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Patterns of variation in the biochemical composition of *Mesopodopsis slabberi* (Van Beneden, 1861) (Crustacea: Mysidacea)

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ABSTRACT

In order to assess the patterns of variation in the biochemical composition of *Mesopodopsis slabberi* (Van Beneden, 1861) in a temperate estuary, we determined the protein, carbohydrate, chitin and lipid contents of freshly caught immature individuals (juveniles), adult males, and adult females during two annual cycles. Statistical analysis (anova) revealed significant differences in the biochemical components between months and development stages for the months analysed. All of the biochemical components showed significant differences between months. Environmental and trophic conditions were the main processes determining seasonal patterns of variation in biochemical composition. The results of complementary experiments carried out to estimate the effects of starvation on biochemical composition showed that the process of starvation consumed mostly carbohydrates, and also that these compounds, together with lipids, were the most affected during the refeeding period. Our findings also indicate that all body reserves were almost fully replenished during refeeding, except for carbohydrates and lipids in adult females and immature individuals (juveniles) and protein content in immature individuals (juveniles). These results indicate that responses to fasting in *M. slabberi* may be dependent on individual development stages, an observation that is corroborated by the significant differences in the biochemical components observed between development stages for the months analysed. Reproduction was also found to influence patterns of variation in biochemical composition.

Keywords: Biochemical composition, estuaries, *Mesopodopsis slabberi*.

RESUMEN

Modelos de variación de la composición bioquímica de* Mesopodopsis slabberi *(Van Beneden, 1861) (Crustacea: Mysidacea)

Para evaluar los modelos de variación de la composición bioquímica de Mesopodopsis slabberi *(Van Beneden, 1861) en un estuario templado, determinamos los contenidos en proteínas, hidratos de carbono, quitina y lípidos de individuos inmaduros (juveniles) recién cogidos, y de machos y hembras adultos, durante dos ciclos anuales. Los análisis estadísticos (anova) revelaron, para los meses analizados, diferencias significativas en las composiciones bioquímicas entre los meses y los estados de desarrollo. Todos los componentes bioquímicos mostraron diferencias significativas entre meses. Los factores ambientales y tróficos fueron los principales procesos que determinaron los modelos estacionales de variación en la composición bioquímica. Los resultados de los experimentos complementarios, realizados para determinar los efectos de la inanición sobre la composición bioquímica, pusieron de manifiesto que los procesos de inanición consumen, fundamentalmente, hidratos de carbono, y, también, que estos compuestos, junto con los lípidos, son los más afectados*

durante el periodo de vuelta a la alimentación. Nuestros descubrimientos indicaron también que todas las reservas corporales son casi completamente repuestas durante la vuelta a la alimentación, excepto los hidratos de carbono y los lípidos en las hembras adultas y en los inmaduros (juveniles), y el contenido en proteínas en los juveniles. Estos resultados indican que las respuestas al ayuno de M. slabberi *podrían depender del estado de desarrollo individual, observación que es corroborada por las diferencias significativas observadas, en los componentes bioquímicos y para los meses analizados, entre los distintos estados de desarrollo. Se encontró también que la reproducción tenía influencia sobre los modelos de variación de la composición bioquímica.*

Palabras clave: *Composición bioquímica, estuarios,* Mesopodopsis slabberi.

INTRODUCTION

Mesopodopsis slabberi (Van Beneden, 1861) is an abundant species in the Mondego estuary (western Portugal), and plays an important ecological role in its pelagic communities, making a substantial contribution to the pelagic standing stock (Azeiteiro and Marques, 1999; Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2002; Azeiteiro, Ré and Marques, 2002). As in other estuaries, due to its abundance, *M. slabberi* has a key position in the energy flow from supra-benthic to plankton and nekton communities (Azeiteiro *et al.*, 2000; Azeiteiro, Fonseca and Marques, 2001; Azeiteiro, Fonseca and Marques, 2002; Azeiteiro *et al.*, 2001).

The *M. slabberi* population reproduces continuously in the Mondego estuary, exhibiting clear spatial and temporal (tidal and seasonal) migration patterns (Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2002). This type of migration has been described in other estuaries (Collins and Williams, 1982; Webb and Wooldridge, 1990) for *M. slabberi,* and may have underlying, salinity-related, reproductive significance (Greenwood, Jones and Greenwood, 1989).

Studies on seasonal variations in body composition are intended to yield information that may be useful in understanding the ecophysiology of a population (Lehtonen, 1996). Seasonal changes in the biochemical composition of crustaceans over their life cycles may reflect metabolic activity related to the nutritional cycle and/or synthesis of reserves in reproductive cells (Azeiteiro, Fonseca and Marques, 2001; Pastorinho *et al.*, 2003).

The main objectives of the present study on *M. slabberi* biochemical composition were: 1) to identify temporal patterns of variation; 2) to identify developmental stage patterns of variation; 3) to analyse interannual variation; and 4) to investigate the impact of starvation.

MATERIALS AND METHODS

Study site and environmental conditions

Study site

The Mondego River, on the western coast of Portugal, drains a hydrological basin of approximately 6670 km², and its estuary (40º 08' N, 8º 50' W) has an area of 3.3 km² and a volume of 0.0075 km³. The Mondego estuary consists of two arms, north and south, separated by Murraceira Island. The two arms become separated in the area upstream from the estuary, at about 6.5 km from the sea, and converge again near the mouth. The tidal range is 0.35 to 3.3 m, with an average freshwater discharge of 8.5×109 m³ s^{-1}, and the average residence time is 2 days in the north arm and 9 days in the south arm. The south arm is only 2-4 m deep, and is almost silted up in the upstream areas. Consequently, the water circulation in the south arm depends basically on the tides and, in a very limited and seasonal way, on freshwater discharge from a tributary, the Pranto River, controlled by a sluice (Azeiteiro and Marques, 2000; Vieira *et al.*, 2002).

Environmental conditions

The estuarine seasonal cycles of temperature and salinity (Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002) are similar to those in many temperate ecosystems (Valiela, 1995). Seasonally varying river flow decreases salinity in the winter months, and light determines the highest temperatures in the summer months (Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002). Seasonal dynamics of phytoplankton are determined by light, depth of vertical mixing, nutrient supply, and grazing pressure, and present a bimodal cycle (Azeiteiro and

Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002).

Sampling programme

Suprabenthic (Azeiteiro and Marques, 1999; Azeiteiro, Ré and Marques, 2002) and crepuscular plankton samples (Azeiteiro, Jesus and Marques, 1999) were collected monthly, from June 1996 to July 1997, and from September 1999 to June 2000 (Pastorinho *et al.*, 2003), in an area where specimens of *M. slabberi* were known to be most abundant, according to previous population dynamics studies (Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2001, 2002). Always following the same sequence, quantitative samples of mysids were taken during spring tides, from sub-surface waters (60 cm diameter and 335 µm mesh net, sub-superficial tows) at high tide and sunset, and with suprabenthic tows (50 cm diameter and 500 µm mesh net) for low-tide diurnal samples.

Laboratory procedures

All samples were transported to the laboratory under good conditions (in water) insofar as temperature (approx. 4 ºC) and oxygen were concerned, within a maximum of 2 h of collection. In the laboratory, they were left to evacuate their guts in filtered seawater (for 12-24 h) prior to freezing for biochemistry. The organisms were classified and separated into immature individuals (juveniles) and adults, females and males (Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2002). Samples from each group were lyophilised, weighed, and kept at –30 ºC; smaller portions of this material were later weighed and used for the determination of each biochemical constituent (analytical replicates) (Azeiteiro *et al.*, 2000; Azeiteiro, Fonseca and Marques, 2001, 2002; Azeiteiro *et al.*, 2001).

Biochemical analyses

Proteins

Lyophilised material was homogenised in the proportion of 0.5 mg to 3 ml of pure water (Micropur) into 10 ml test tubes. The water-soluble protein content was analysed (n = 5-6 subsamples) using the method described in Lowry *et al.* (1951), as modified by Fernandes *et al.* (1994).

Carbohydrates

Samples were separated for analysis, following essentially the same procedure as for proteins. The homogenates were analysed (n = 4-5 subsamples) with the method devised by Raymont, Austin and Linford (1964), as described in Båmstedt (1976) and Omori and Ikeda (1984), using 1 ml of a 5 % phenol solution and 5 ml of concentrated sulphuric acid.

Chitin

The analysis was performed using the Båmstedt (1976) method for dried homogenised material (n = 4-5 subsamples).

Total lipids

The analysis was performed following the method described by Lehtonen (1996). Approximately 15 mg of lyophilised material was weighed and homogenised in 0.5 ml of a chloroform:methanol (2:1) solution, and then centrifuged for 30 seconds. The precipitate was washed with 0.5 ml chloroform:methanol (2:1) and centrifuged for 30 seconds. Twenty per cent volumes of 0.9 % NaCl solution were added to the chloroform:methanol (2:1) solution from both washes, and centrifuged. The chloroform phase containing the dissolved lipids was placed into tarred cups, and the solvent evaporated. The cups were then weighed, and the weight of the lipids calculated from triplicate subsamples.

Effects of starvation on biochemical composition

Experiments on the effects of fluctuating food availability were designed to provide information, on a complementary basis (specimens collected during one sampling in spring 2000), about the im-

pact of starvation on the biochemical composition of *M. slabberi* (n = 3 subsamples). Freshly caught animals (transported to the laboratory in estuarine water where they were left to evacuate their guts in filtered estuarine water) were classified and separated into groups of immature individuals (juveniles), adult males and adult females. Individuals were captured and frozen in liquid nitrogen after each of the following phases: Phase I: 0 hours - starting fasting period; Phase II: 24 hours - end fasting period; Phase III: 36 hours - end refeeding (*ad libitum*) period. These experiments were designed according to Webb, Perissinotto and Wooldridge (1987).

Data analysis

For the statistical analysis of data, we used a repeated measurements anova (Zar, 1996), pairing data on the development stages (immature-juveniles, adult males and adult females) according to sampling months. Data was log-transformed prior to the anova, in all cases (Zar, 1996).

RESULTS

Data are presented in table I for both annual cycles. The data relative to 1996-1997 has already been published (Azeiteiro *et al.*, 2000; Azeiteiro, Fonseca and Marques, 2001; Azeiteiro *et al.*, 2001). Anova results are summarised in table II.

Protein

The primary body component, throughout the year, constituted on average more than half of the dry weight. Protein contents (% of dry weight) varied from 58.2 % - 74.8 % in 1996-1997 and 64.8 % - 78.6 % in 1999-2000, for immature individuals (juveniles); for adult females, from 61.7 % - 83.8 % in 1996-1997 and 65.3 % - 76.3 % in 1999-2000; and for adult males, from 58.1 % - 78.7 % in 1996-1997 and 63.2 % - 78.7 % in 1999-2000. In 1996-1997, with the exception of the winter season, the protein content in immature individuals (juveniles) was lower than in adult females and adult males. Immature individuals (juveniles) showed a small decrease in protein proportion in November, followed by a slow increase up to early May. By the end of May, a new and more accentuated decrease was observed, followed again by a slow increase. Adult female protein contents exhibited continual variation throughout the year, with the lowest and highest values in May, at the beginning and at the end of this month, respectively. A small decrease was also registered in November. Adult males never showed a great deal of variation in protein proportion, except for a clear decrease in December and a smaller one June. With the exception of December 1999 and February 2000, the protein contents followed a similar pattern in adult males, adult females and immature individuals (juveniles), with a decrease towards the winter months, and reaching the highest values for adult males and adult females in May and for immature individuals (juveniles) in September. Protein contents were significantly different ($p < 0.05$; $P = 0.0204$; $\alpha = 0.05$) from one month to another.

Carbohydrates

Immature individuals (juveniles) consistently presented a higher carbohydrate proportion than adult females or adult males, which showed a similar variation. In fact, carbohydrate proportion (% of dry weight) varied from 6.24 % - 16.12 % in 1996-1997 and 8.3 % - 13.6 % in 1999-2000, for immature individuals (juveniles); from 4.86 % - 28.99 % in 1996-1997 and 6.0 % - 8.4 % in 1999-2000 for adult females; and from 5.2 % - 30.89 % in 1996-1997 and 6.0 % - 12.0 % in 1999-2000, for adult males. For both annual cycles studied, immature individuals (juveniles) showed the lowest value in November, and the maximum at the end of May. Regarding adult males, the lowest values were observed during winter, with minima in November 1996 and February 2000, and the highest during spring, with maxima in October and the beginning of May for both annual cycles studied. Adult females followed such a pattern, as well, in the months of October and May, when the highest values were registered. Carbohydrate contents were significantly different ($p < 0.05$; $P = 0.0065$; $\alpha = 0.05$) between months and between development stages ($p < 0.05$; $P = 0.0000017$; $\alpha = 0.05$) for the analysed months.

Table I. Biochemical composition (% dry weight) of *M. slabberi* in the Mondego estuary

Immature individuals (juveniles)

		Proteins		Carbohydrates		Chitin		Lipids	
	Sampling times	Average	StDev	Average	StDev	Average	StDev	Average	StDev
1996-1997	October	74.8	7.9	10.32	1.11			42	
(Azeiteiro,	November	64.3	2.4	6.24	2.16		0.00	10	
Fonseca and	December	67.7	4.9	11.94	1.64	4.06	0.96	12	
Marques, 2001)	March	69.7	2.9	11.81	2.80	3.14	1.67	11	
	May (9)	72.0	3.1	11.04	4.17			7	
	May (24)	58.2	4.7	16.12	3.40	0.98	0.34	22	
	June	61.0	3.3	10.93	1.76	0.75	0.15	15	
	July	70.3	1.7	8.64	2.35	0.61	0.19	42	
1999-2000	September	78.6	4.8	11.9	1.2	3.2	0.37	10.3	0.32
	October	75.3	3.5	11.0	2.4	4.4	0.69	12.1	0.37
	November	70.2	2.7	8.3	3.3	4.9	0.54	13.5	0.48
	December	65.7	5.3	10.4	3.9	5.2	0.71	12.9	0.45
	January	64.8	3.1	9.9	4.3	6.9	0.78	12.7	0.62
	February	65.2	4.5	10.1	4.1	5.3	0.66	13.7	0.73
	April	68.1	4.2	11.2	3.5	4.9	0.68	13.5	0.54
	May	72.5	4.0	13.6	2.2	4.3	0.43	12.4	0.39
	June	73.1	3.3	12.9	2.6	4.3	0.41	13.0	0.34

Adult males

		Proteins		Carbohydrates		Chitin		Lipids	
	Sampling times	Average	StDev	Average	StDev	Average	StDev	Average	StDev
1996-1997	October	70.0	5.2	30.89	39.18				
(Azeiteiro,	November	73.8	6.6	5.20	0.66	2.25	0.52	15	
Fonseca and	December	58.1	1.9	8.39	1.24	7.00	2.58	11	
Marques, 2001)	March	74.0	2.5	6.79	1.75	3.64	1.83	10	
	May (9)	74.4	4.5	9.54	2.42			8	
	May (24)	75.2	3.7	8.73	1.06	0.91	0.23	9	
	June	70.8	3.1	8.57	2.56	0.48	0.16	8	
	July	78.7	1.5	8.64	1.42	0.71	0.23	10	
1999-2000	September	76.5	1.1	9.9	5.2	4.7	0.17	8.4	0.52
	October	73.4	1.5	12.0	3.7	7.0	0.49	12.3	0.28
	November	68.1	3.2	8.9	4.1	5.7	0.18	18.5	0.63
	December	71.5	2.1	7.1	2.4	4.8	0.42	15.9	0.24
	January	66.7	4.8	6.9	6.3	7.2	0.28	13.2	0.33
	February	63.2	3.3	6.0	4.8	5.4	0.26	16.7	0.51
	April	69.7	1.8	7.4	7.7	3.1	0.47	19.3	0.37
	May	78.7	3.2	9.6	2.3	2.8	0.36	17.3	0.44
	June	71.1	2.6	8.7	4.3	2.0	0.24	18.8	0.32

Adult females

		Proteins		Carbohydrates		Chitin		Lipids	
	Sampling times	Average	StDev	Average	StDev	Average	StDev	Average	StDev
1996-1997	October	74.6	7.6	28.99	31.47				
(Azeiteiro,	November	61.7	3.0	12.35	4.14	3.66	1.04	25	
Fonseca and	December	70.3	5.1	6.02	0.55	5.44	2.72	19	
Marques, 2001)	March	67.6	2.9	7.24	2.69	2.11	0.55	43	
	May (9)	83.8	3.9	11.06	2.21			14	
	May (24)	61.8	2.7	9.40	1.60	0.81	0.21	10	
	June	69.4	6.5	4.86	1.53	0.66	0.22	11	
	July	72.0	2.1	9.27	1.78	0.66	0.24	10	
1999-2000	September	73.8	3.1	6.8	3.2	3.5	0.24	16.7	0.17
	October	70.5	5.2	8.4	5.1	4.5	0.31	18.5	0.31
	November	68.9	4.7	6.2	2.7	9.3	0.62	15.4	0.57
	December	65.3	5.2	6.5	3.8	11.0	0.54	15.0	0.48
	January	67.6	3.8	6.0	4.9	10.9	0.81	14.5	0.73
	February	71.3	2.9	6.3	6.2	9.3	0.42	15.3	0.39
	April	74.1	3.4	6.0	5.4	7.3	0.38	15.6	0.36
	May	76.3	5.1	8.4	2.8	6.3	0.47	14.1	0.53
	June	73.8	4.6	8.1	3.1	5.9	0.65	14.4	0.41

% Dry weight

Table II. Anova of the biochemical composition of *M. slabberi*

	Proteins			Carbohydrates			Lipids			Chitin		
	F	P-value	F crit	F	P-value	F crit	F	P-value	F crit	F	P-value	F crit
Development stage	0.211	0.81200	3.634	34.2356	0.0000017	3.6337	4.3868	0.0303	3.6337	8.8609	0.0026	3.6337
Month	5.415	0.00204	2.591	4.2741	0.0065139	2.5911	1.0557	0.4381	2.5911	2.9838	0.0299	2.5911

Chitin

The variation of chitin proportion in 1996-1997 was basically similar in immature individuals (juveniles), adult females, and adult males, with the average varying from 0.48 % - 7 %. The highest registered values occurred in December, and the lowest during spring. In the annual cycle 1999-2000, with the exception of October, the variation pattern of chitin was similar both in adult males and immature individuals (juveniles), with a maximum in January. Adult females also presented maximum values in winter months. Chitin contents were significantly different ($p < 0.05$; $P = 0.0299$; $\alpha = 0.05$) between months and between development stages ($p < 0.05$; $P = 0.0026$; $\alpha = 0.05$) for the study period.

Lipids

Lipid contents (% of dry weight) for immature individuals (juveniles) varied from 7 % - 42 % in 1996-1997 and 10.3 % - 13.7 % in 1999-2000; from 10 % - 43 % in 1996-1997 and 14.1 % - 18.5 % in 1999-2000, in adult females; and from 8 % - 15 % in 1996-1997 and 8.4 % - 18.8 % in 1999-2000, in adult males. In November, immature individuals (juveniles) presented the highest values, followed by a clear decrease, with low values recorded during the winter and early spring, with another increase in June. Adult female lipid proportions peaked in March. In adult males, there was almost no variation throughout the year, although slightly higher values could be found during winter. After October 1999, adult females and immature individuals (juveniles) presented similar variation pattern for lipids. Adult females showed a maximum in October and a minimum in July 2000. Lipid contents were significantly different ($p < 0.05$; $P = 0.0303$; $\alpha = 0.05$) between months.

Effects of starvation on biochemical composition

The results of the complementary experiments carried out to estimate the effects of starvation on biochemical composition showed that the process of starvation mostly consumed carbohydrates. Moreover, these compounds, together with lipids, were the most affected during the refeeding period (figure 1), when the accumulation of carbohydrate and lipids in adult females and immature individuals (juveniles) did not reach the initial levels. These results indicate that responses to fasting in *M. slabberi* may be dependent on individual organisms' developmental stages.

Anova results of the interannual analysis showed that only chitin contents showed a significant difference ($p < 0.001$) between the two study periods.

DISCUSSION

M. slabberi presents significant temporal variation in its biochemical composition (Azeiteiro, Fonseca and Marques, 2001). Protein, lipid, and carbohydrate accumulation cycles show their maxima in spring, apparently in relation to individuals' nutritional state and breeding cycle (Pastorinho *et al.*, 2003), as well as temperature variations (Clarke, 1977). The maxima of carbohydrates, lipids and proteins occur in late autumn (November) and spring. The species accumulates lipids during the first phytoplankton blooms in February (Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002). Females show a lipid accumulation peak in September, October and March; juveniles in November, after a massive recruitment period occurring in late summer/autumn and spring/early summer (Azeiteiro, Jesus and Marques, 1999), and the third minor phytoplankton bloom in September and October (Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002). Females restore their body mass after the main recruitment periods (Azeiteiro, Fonseca and Marques,

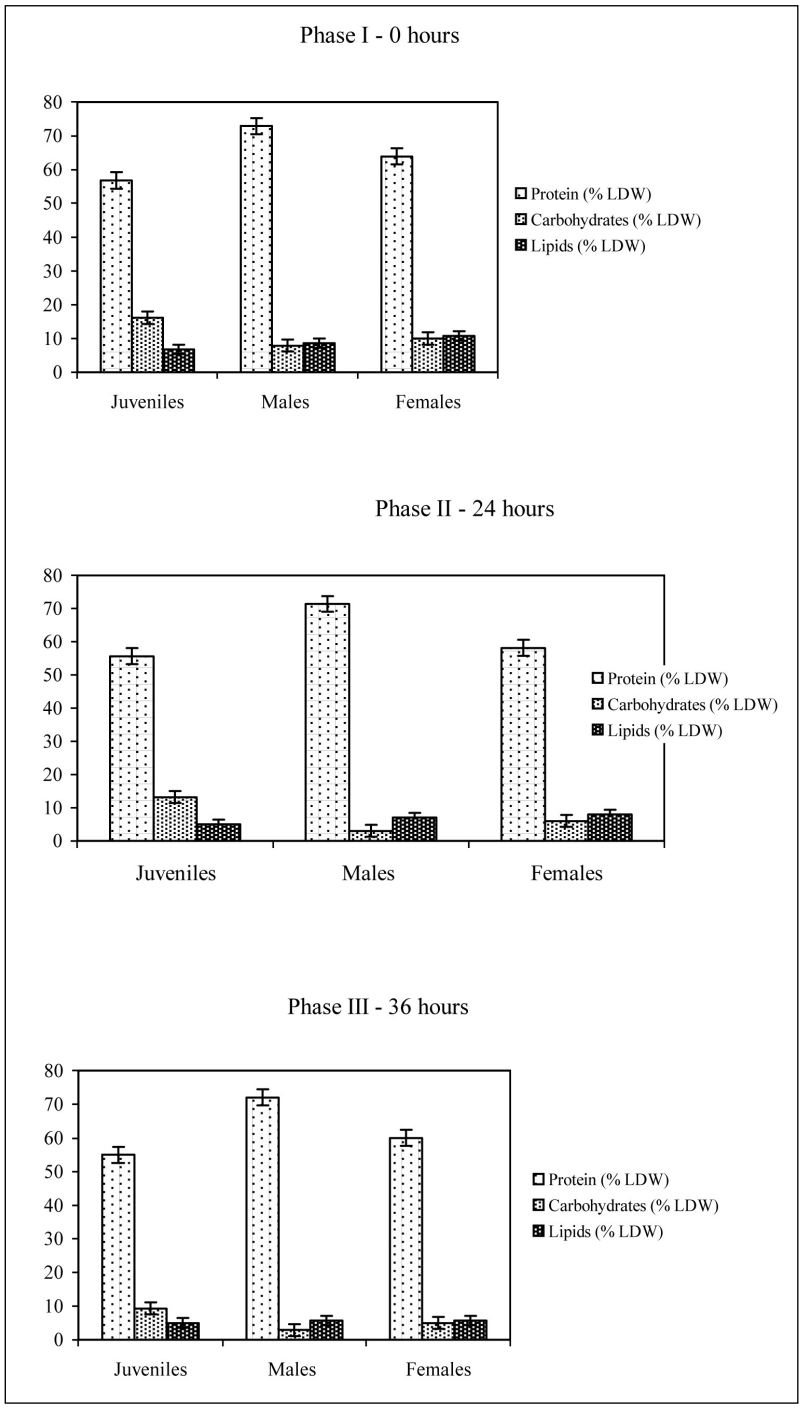

Figure 1. Variation of the biochemical composition of *M. slabberi* as a function of food supply fluctuations after a starvation period. Phase I: 0 hours - start fasting period; phase II: 24 hours - end fasting period; phase III: 36 hours - end refeeding period

2001). Gender influence can be noticed as a lack of synchronisation in reaching spring maxima.

Our results were basically consistent with the literature on Crustacean biochemical composition (Raymont, Austin and Linford, 1964, 1968; Båmstedt, 1975, 1976, 1978; Lehtonen, 1996), with particular regard to other mysid species (e.g., *Boreomysis arctica* Kroyer and *Neomysis integer* Leach). Although there are few studies on mysids whose findings are comparable, our results were also consistent with data obtained for a number of benthic and suprabenthic peracarid species (Raymont, Austin and Linford, 1964, 1968; Båmstedt, 1975, 1976, 1978; Johnson and Hopkins, 1978; Omori and Ikeda, 1984; Chigbu and Sibley, 1996; Lehtonen, 1996).

Proteins were, in proportion, the main body component throughout the year, and also the most

stable (although we did find significant differences between months). In other groups (Moss and Lawrence, 1972; Ortega, López de Pariza and Navarro, 1984), fluctuations in protein content are related to the tissue water content, which suggests that protein variation reflects seasonal fluctuations in the hydratation level of the tissues (Ortega, López de Pariza and Navarro, 1984). Minimum values in winter months, coinciding with adverse environmental conditions, suggest that weight loss during this period (Azeiteiro, Jesus and Marques, 1999) may also be sustained by proteins (Azeiteiro, Fonseca and Marques, 2001).

Carbohydrates showed significant temporal variability, indicating both rapid accumulation and depletion (i.e., easily accessible reserve). Despite a great accumulation in autumn (October), carbohydrate contents tended to decrease towards the winter (starvation period), particularly in the case of adult females in 1996-1997. The results of the complementary experiments carried out to examine the effects of starvation on biochemical composition showed that the process of starvation consumed mostly carbohydrates. Moreover, carbohydrates and lipids were the components most affected during the refeeding period, when the accumulation of carbohydrate and lipids in adult females and immature individuals (juveniles) did not reach starting levels (a longer period of refeeding may yield different data). Such findings indicate that the responses to fasting in *M. slabberi* may be dependent on the development stage of individual organisms (the annual cycle anova results gave us significant differences on carbohydrate contents between development stages for the months analysed). For both annual cycles, immature individuals (juveniles) showed the lowest value in November, and the highest at the end of May. With regard to adults, we also observed the lowest values during winter and the highest ones during spring, with maxima in October and May for both annual cycles, following the month with high chlorophyll *a* concentration (Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002).

The variation in chitin proportions showed significant differences between months and development stages for the months analysed. The highest values were observed in the winter months and the lowest during spring, although this might not reflect an absolute variation in chitin, but rather a changing relationship between the surface and volume of the individuals as they pass from a winter starvation period to one of high food availability (Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2001; Azeiteiro and Marques, 2000; Bacelar-Nicolau *et al.*, 2003; Vieira *et al.*, 2002).

Lipid variation is a function of metabolism and reproductive strategy (Pastorinho *et al.*, 2003), depending therefore on the species's life cycle (Pastorinho *et al.*, 2003). In fact, many life-span traits of aquatic invertebrates depend on investments in lipids (Lehtonen, 1996; Ohman, 1997). However, although lipid contents showed significant differences between months, they did not do so between development stages.

In *M. slabberi*, as in other crustaceans, biochemical changes result from metabolic aims in relation to the nutritional cycle and/or synthesis of reserve products related to reproduction (the latter more noticeable in females) (Azeiteiro, Fonseca and Marques, 2001). Therefore, both environmental and trophic conditions, as well as reproduction, should play an important role in determining seasonal changes in biochemical composition (Azeiteiro, Fonseca and Marques, 2001; Pastorinho *et al.*, 2003). Marques *et al.* (1994) established that the prevailing conditions in the Mondego estuary, namely eutrophication, could result in the development of opportunistic adaptive strategies among invertebrate species. The high specific production of the *M. slabberi* population (Azeiteiro, Jesus and Marques, 1999; Azeiteiro, Fonseca and Marques, 2001, 2002); the fact that in *M. slabberi* biochemical changes result mainly from metabolic aims related to environmental conditions and the nutritional cycle (significant temporal differences were found in all biochemical components) and, less importantly, to reproductive synthesis; along with the observation (Pastorinho *et al.*, 2003) that all sampled material has shown the presence of reproductive cells (oocytes) in mature ovigerous, brood-pouched or resting mature females, having the capability (due to their developmental status and qualitative carbohydrate and lipid contents, or reserve material) for continuous reproduction (Pastorinho *et al.*, 2003)–all of this reinforces our conviction that environmental conditions and the nutritional cycle are the determinant conditions in the observed biochemical variation patterns in the Mondego estuarine system. The yearly cycle of *M. slabberi*, like that of other temperate estuarine species, appears

to be a mechanism for coping with seasonal changes in the environment (Johnston and Northcote, 1989).

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